INTRODUCTION

The requirement to retain and analyze polar molecules by HPLC is one that has grown steadily over the last few years, and has been the driving force behind the generation of a range of new stationary phases dedicated to this purpose. The new packings offer an alternative mechanism of interaction that takes place in addition to dispersive interactions generally associated with the more tradition alky silane type packings. Such interactions allow for increased retention and improved peak shape of analytes with polar functionality whether basic or acid in nature. Such packings are not only targeted at the separation of polar analytes but often encompass the application range of the traditional alkyl C18 packings also.

In this technical bulletin, we review columns made with several packings that have been tailored to go beyond the limitations of the traditional C18 packing materials. The columns that we review are listed as follows and are discussed individually throughout the bulletin:

- **AQUASIL C18 - Wettable reversed phase**
- **HyPURITY® ADVANCE - Imbedded polar group phase**
- **Hypercarb® – Porous Graphitic Carbon**
- **PRISM® - Imbedded polar group phase**
- **Fluofix® and Fluophase® - Perfluorinated phases**

Without exception, the phases outlined above achieve quite different chromatographic behavior to the more traditional type C18 packings in that they provide:

- Alternative selectivity
- Increased retention of polar compounds
- The ability to run in highly aqueous mobile phase
- The ability to be used at reduced buffer concentrations

Figure 1 gives an indication of how two of the phases, the PRISM RPN column and the HyPURITY ADVANCE column, can give rise to alternative selectivity for the more polar basic compounds in a test mixture compared to C18. These phases are discussed in greater detail later in the bulletin.
Selectivity
Packings that offer additional modes of interaction give rise to quite different retention behavior and selectivity. In general analytes with the greatest polar functionality will typically show greatest changes in selectivity and retention.

Highly Aqueous Mobile Phases – Increased Retention
The inclusion of polar functionality to the stationary phase also increases the wetting characteristics of the packing in highly aqueous mobile phases (100% aq). Several of the packings outlined in this bulletin can be run in 100% aqueous mobile phase conditions and show no tendency towards phase collapse or folding. Phase collapse is often seen for alkyl C18 packings where a small amount of organic solvent (1-5%) is generally required in the mobile phase to help wet the C18 surface and prevent phase collapse. See separate technical bulletin #TB99-01 for further explanation of phase collapse/folding.

Reduced Buffer Concentrations – Increased MS Sensitivity
Several of the phases outlined in this report are also shown to maintain chromatographic performance when very low concentrations of buffers are used. Low buffer concentrations offer the reward of increased sensitivity for MS. This is an important consideration when trying to identify trace quantities of a drug compound or impurity that may normally disappear into the noise of the base line of the MS response.

Further Information
A brief review of typical questions that are often raised concerning these stationary phases is given in the following pages. For more in-depth information on each product please request the individual Product Bulletins (as highlighted for each product) from Thermo Hypersil-Keystone Technical Support.
How does retention of polar analytes compare with that of traditional C18 alkyl bonded phase?

The AQUASIL C18 phase was designed for the reversed phase separation of polar molecules. It has a high concentration of C18 groups as well as hydrophilic sites that help to provide retention of highly polar water soluble compounds (Figure 2), but offers the added benefit of nearly twice the retention of polar compounds when compared to BetaBasic® 18.

AQUASIL C18 retention is comparable to a traditional C18 when run in mobile phase with high organic.

Can I use the AQUASIL C18 in 100% aqueous mobile phase?

Yes! The extra polar character associated with AQUASIL C18 allows the use of mobile phases without any organic component, i.e. 100% aqueous (Figure 3). Traditional C18 packings with high carbon loads require at least 3 to 5% organic component in the mobile phase to prevent phase collapse (folding). During this process, the retention and selectivity of the phase is lost and the column must be regenerated using a pure organic solvent wash. AQUASIL C18 is immune to this folding due to its unique polar functionality.

Can I run low pH applications with reduced buffer concentration?

When used in high concentrations, buffers (e.g. trifluoroacetic acid) can cause MS ion source supression and consequently can reduce sensitivity. The choice of column used is of key importance for LC/MS applications since the quality of the C18 packing and underlying silica can strongly influence the concentration of buffer required. In this example, we show that buffer concentration (TFA) can be reduced to zero concentration without loss in performance when using AQUASIL C18 (Figure 4). It is generally good practice to buffer your mobile phase, but with the AQUASIL C18 column, very low concentrations can be used.
PRISM® RP and PRISM RPN Columns

PRISM was developed to offer alternative selectivity to traditional alkyl C8 or C18 packings. It has unique chemistry that involves the incorporation of polar functional groups near the silica surface. These polar functional groups also form part of the alkyl chain that is responsible for the primary mode of interaction between the stationary phase and the analyte. Imbedded polar groups allow for a secondary or mixed mode type of interaction to take place, which in turn leads to a quite different retention behavior for polar analytes.

PRISM RP & PRISM RPN
Polar imbedded groups with C12 chemistry

Are PRISM columns available in both endcapped and non-endcapped versions?
Yes. Both columns offer alternative selectivity and excellent peak shape for basic compounds. The non-endcapped PRISM RPN phase gives different selectivity for moderately polar and basic analytes such as tricyclic antidepressants (Figure 5).

The endcapped PRISM RP phase gives slightly different selectivity and offers improved peak shape for acidic analytes in particular.

Can the PRISM RP and PRISM RPN columns be run in 100% aqueous mobile phase?
Yes. In order to maximize retention of many very polar compounds, it is usual practice to reduce the percentage of the organic component in the mobile phase. Packings, such as PRISM RP and PRISM RPN, which contain imbedded polar groups near the surface of the silica, allow the organic component of the mobile phase to be reduced to zero, therefore maximizing the possibility of retention of highly solvated polar molecules (Figure 6).

How does trifluoracetic acid (TFA) concentration affect resolution?
Figure 7 shows the separation of three simple Angiotensin peptides. The resolution is shown to improve significantly for peaks 1 & 2 as the concentration of TFA decreases to 0.01%.

Increasing the TFA concentration is generally thought to increase the hydrophobic properties of basic analytes when in ionic state. It does this by displacement of water molecules with TFA counterions. An increase in retention is observed as the concentration of TFA is increased but also a loss of resolution. Even at low concentrations of TFA, the polar PRISM packing can interact with the analyte to provide increased resolution. Changes in pH of the different concentrations can also affect resolution.

For more information, please request Product Bulletin PB01-18.
HyPURITY ADVANCE was developed to offer alternative selectivity to the traditional alkyl C8 or C18 packings. It has unique chemistry that involves the incorporation of polar functional groups (different chemistry than PRISM® phases) near the silica surface. An alkyl C8 group is responsible for the primary mode of interaction between the stationary phase and the analyte. The imbedded polar groups allow a secondary or mixed mode type of interaction to take place, which leads to quite a different retention behavior and improved peak shape for many of the more polar analytes.

**Should I expect increased or decreased retention of my polar analytes?**

Basic compounds generally show reduced retention on HyPURITY ADVANCE columns, especially at pH 2-5 where the imbedded polar group carries a positive charge and can repel approaching similarly charged groups (Figure 8).

Acidic compounds conversely are retained slightly longer. Both effects contribute to quite different retention behavior compared to traditional C18 silicas with the added advantage that excellent peak shapes are obtained for acidic, basic and neutral compounds. Where ionization of the analyte has been suppressed, interaction takes place via dispersive interactions with the C8 group, and also by dipole-dipole interactions between polar groups on the stationary phase and the polar groups on the analyte. This gives rise once again to alternative selectivity (Figure 9).

**Can I run HyPURITY ADVANCE columns in 100% aqueous mobile phase?**

Yes. The HyPURITY ADVANCE phase shows a complete absence of folding or phase collapse when run in 100% aqueous conditions.

Figure 10 shows an example where the HyPURITY ADVANCE column has been used to analyze several polar catecholamines. The mobile phase consists of 20mM phosphate buffer, pH 7.5. The analysis is run in complete absence of any organic modifier.

**Are there other applications available that have been run on the HyPURITY ADVANCE column?**

Yes. Please request the HyPURITY Applications Booklet and HyPURITY Product Guide for further information on selectivity, stability, compatibility with MS and speed of analysis.
Fluorinated Phase Columns

Fluorinated stationary phases offer a new approach to stationary phase selectivity. Fluorine atoms with their highly electronegative character offer alternative molecular interactions by which polar analyte retention can take place. Three different packings are available; see Figure 11 to compare selectivity.

Does the fluorine chemistry give rise to alternative chromatographic selectivity?

The fluorine chemistry often behaves in a similar manner to a traditional alkyl bonded packing. Where an analyte has some polar functionality, fluorinated packings can often give quite different selectivity. This is particularly the case for fluorinated or chlorinated compounds. Fluofix and Fluophase columns have also been shown to give excellent results on non-halogenated compounds such as lipids, surfactants, taxanes, catechins and many other polar compounds with carboxyl or nitro groups.

Figure 11 shows how improved resolution can be obtained using Fluophase RP and Fluofix when compared to analysis of the same compounds using BetaBasic 18. Similar reports have been observed for isomers or compounds closely related in structure.

Can I use 100% aqueous conditions with fluorinated phases?

Resistance to folding is observed for both the Fluofix and Fluophase RP. Fluophase PFP has shown some tendency to fold under 100% aqueous conditions and for this reason it is recommended that at least 5% of the mobile phase composition should contain organic solvent (Figure 12).

What different fluorinated phases are available?

Fluofix 120E is a fluorinated branched-chain hexyl phase on 120Å silica. Fluophase RP and Fluophase WP are fluorinated straight-chain hexyl phases on 100Å and 300Å silica, respectively. Fluophase PFP is a pentafluorophenyl phase on 100Å silica.

For more information, please request Product Bulletin PB01-11.
Porous graphitic carbon has unique properties as a stationary phase and now often provides solutions to what might be considered ‘problem HPLC separations’. Two such problem areas are:

1. the retention and separation of very polar analytes not normally retained on C18 packings.
2. the separation of structurally similar compounds, such as geometric isomers and diastereomers, not always separated on C18 silica.

Figure 13 shows how retention behavior changes for phenol and some polyhydroxybenzenes versus percent organic modifier in the mobile phase. Note how the retention order differs for both Hypercarb and Hamilton PRP:

- **Hypercarb**: 1,3,5 trihydroxybenzene > 1,2 dihydroxybenzene > phenol.
- **PRP-1**: Phenol > 1,2 dihydroxybenzene > 1,3,5 trihydroxybenzene

(Note: under these conditions the two polyhydroxylbenzene analytes are typically not retained on C18 packings.)

Figure 14 shows retention of several very polar pharmaceutical compounds not normally retained on alkyl C18 silica.

What other benefits does Hypercarb offer over traditional alkyl bonded silica packings?

- Enhanced selectivity for closely related compounds
- Retain highly polar compounds very strongly
- Stable across the pH range 1-14
- Can be used with a wide range of solvents from 100% aqueous to 100% hexane or methylene chloride

The flexibility of solvent choice is demonstrated in Figure 15 where polyethoxylated alcohols and phenols are separated on a single Hypercarb column. It is common practice to have to use two columns for this analysis – a C18 for the reverse phase separation of the more polar analytes and a normal phase column for the very hydrophobic analytes.